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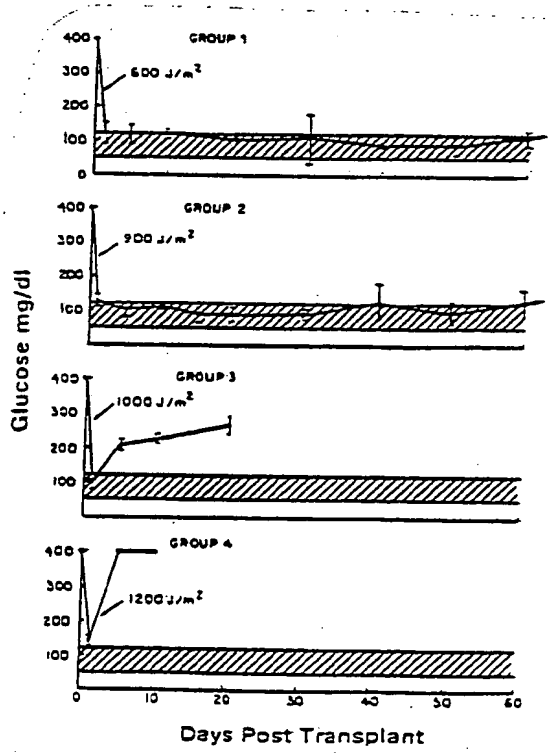
(54) Title: PROCESSES FOR DEVELOPMENT OF ACCEPTANCE OF TRANSPLANTED ORGANS AND TISSUES

(57) Abstract

A patient's acceptance of transplanted organs and tissues may be enhanced by treating donor specific blood with a suitable dose of ultraviolet radiation for an appropriate period of time, transfusing the irradiated blood into the patient during a suitable pretransplantation time period and then transplanting the organ or tissue into the subject. Acceptance of transplanted organs and tissues can also be enhanced by treating the organs or tissues to be transplanted with a suitable dose of ultraviolet radiation for an appropriate period of time and then transplanting the organ or tissue. Suitably irradiated donor specific blood, organs and tissues are preferred for use in transplantation.



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# PROCESSES FOR DEVELOPMENT OF ACCEPTANCE OF TRANSPLANTED ORGANS AND TISSUES

The invention described herein was made in the course of work under grant numbers HL 14799 and AM 30468 from the National Institutes of Health, United States Department of Health and Human Services, U.S.A.

## SUMMARY OF THE INVENTION

The acceptance of transplanted organs or tissues in a subject may be enhanced by treating donor specific blood with a suitable dose of ultraviolet radiation for an appropriate period of time, transfusing the irradiated blood into the subject during a suitable period of time prior to transplanting the organs or tissues and then transplanting the organs or tissues into the subject.

The acceptance of transplanted organs or tissues in a subject may also be enhanced by treating the organs or tissues to be transplanted with a suitable dose of ultraviolet radiation for an appropriate period of time and then transplanting the organs or tissues into the subject.

Suitably irradiated donor specific blood, organs and tissues are preferred for use in surgical transplantation.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Percentage of graft survival in ACI diabetic recipients of islet allografts. Group I (O) transfused Lew UV irradiated blood and Lew islet allografts, Group II (Δ) transfused non-treated Lew blood and Lew islet allografts, Group III (□) control recipients without transfusion and Lew islet allografts, and Group IV (●) transfused as in Group I but transplanted third-party W/F ($RT1^u$) islets.

FIG. 2. The effect of dose of UV irradiation on the MLC stimulatory activity of Lewis dendritic cells (DC). MLC was performed using ACI thoracic duct lymphocytes (TDL) as responders and Lewis rat afferent lymph derived DC as stimulators. Methods used for isolation of dendritic cells have been previously described (20). In brief, abdominal lymph nodes were removed from rats six weeks prior to thoracic duct drainage. Lymph was collected over a 36 hour period and resultant cells were enriched for DC by a high density BSA centrifugation step (44). Resultant light density cells had a population of approximately 70% DC with their distinct morphological appearance (45). These cells were gamma irradiated (1600 rad) prior to UV irradiation and their use in MLC. DC were UV irradiated in open petri dishes while suspended under HBSS with constant stirring with a magnetic bar. Source of UV irradiation was a bank of two Sylvania FS20 lamps which have a flux of $1mW/cm^2$ at 310 nm, (UVX-Radiometer, UV Product, Ca.) measured 10 cm from source. Cells were cultured in triplicate in 96 well microtiter plates in RPMI 1640 containing 100 mg/ml streptomycin and penicillin and supplemented with 10% rat serum. Results represent $(H^3)$=thymidine incorporation after 96 hours of culture including a 16 hour $(H^3)$ thymidine pulse period and are expressed as:

$$\text{Stimulation Index (SI)} = \frac{\text{experimental mean CPM}}{\text{control mean CPM}}$$

FIG. 3. Effect of dose of UV irradiation on islet function after syngeneic transplantation. Lewis rats were made diabetic with i.v. streptozotocin (60 mg/kg) (courtesy of Dr. Dulin, Upjohn, Kalamazoo, Michigan) and used as recipients if blood glucose was 300 mg/dl on three weekly successive measuremens. Lewis islets were isolated using collagenase digestion (46), Ficoll gradient separation (47) and subsequent hand-picking under a stereomicroscope. Isolated islets were suspended in HBSS in petri dishes and irradiated with constant stirring with a magnetic bar. UV source was same as described for DC irradiation. After irradiation, islets were placed into culture at 37°C, 5% $CO_2$ in CMRL 1066 with 10% FCS for 24 hours and transplanted via the portal vein. Group I islets received 800 J/$m^2$ (n=2); Group II islets received 900 J/$m^2$ (n=2); Group III islets received 1000 J/$m^2$ (n=2); and Group IV islets received 1100 J/$m^2$ (n=3). Stippled line represents normal blood glucose range.

FIG. 4. Survival of Lewis UV irradiated and non-irradiated islets in diabetic ACI recipients. ACI rats were made diabetic with i.v. streptozotocin as described before. Lewis islets were isolated as described for syngeneic transplants and were UV irradiated with 900 J/$m^2$ exactly as described previously. UV and non-UV irradiated islets were cultured for 24 hours prior to intraportal transplantation. Rejection of islets was considered to have occurred if blood glucose was 200 mg/dl on two consecutive daily measurements. o---o Lewis islets to ACI without UV irradiation; •---• Lewis islets to ACI with prior UV irradiation.

## DETAILED DESCRIPTION OF THE INVENTION

Throughout this application references are made using arabic numerals to various publications and explanatory matter set forth immediately preceding the claims under the heading "References and Notes." The entire disclosures of these references are hereby incorporated by reference into the present application to provide information concerning the state of the art as of the time the invention was made.

This invention provides a method for enhancing acceptance of transplanted organs or tissues in a subject which comprises treating donor specific blood with a suitable dose of ultraviolet radiation for an appropriate period of time, transfusing the irradiated blood into the subject during a suitable period of time prior to transplanting the organs or tissues into the subject and then transplanting the organs or tissues into the subject.

Although applicable to a wide variety of subjects the invention is primarily intended for use with human patients requiring an organ or tissue transplant. In principle the method may be used with any organ or tissue. Examples of such organs include the kidney, heart, lung, liver and intestine. Tissues may be derived from these organs or may be derived from other body tissues, e.g., bone marrow.

Varying doses of ultraviolet radiation may be employed to irradiate the organ or tissue to be transplanted. The presently preferred dosage is a dose less than about 1000 $J/m^2$. The organ or tissue may be exposed to the ultraviolet radiation for various periods of time. The presently preferred time for exposure to the ultraviolet radiation is a time period greater than about ten minutes, e.g., about twenty minutes.

The irradiated blood is desirably transfused into the subject into whom the organ or tissue is to be transplanted during a suitable period of time prior to the actual transplantation procedure. Preferably, the transfusion with donor specific irradiated blood is performed more than once and at least one week prior to transplantation of the organ or tissue. Presently it is preferred that the subject receive transfusion three times: at three weeks, at two weeks and at one week prior to the transplant. After the subject has received transfusions of suitably irradiated donor specific blood, the organ or tissues is transplanted into the subject using conventional surgical procedures.

An alternative approach to the use of transfused, irradiated donor specific blood to enhance the acceptance of transplanted organs or tissues involves the use of irradiated organs or tissues. Accordingly, this invention also provides a method for enhancing acceptance of transplanted organs or tissues in a subject which comprises treating the organ or tissue to be transplanted with a suitable dose of ultraviolet radiation for an appropriate period of time and then transplanting the irradiated organ or tissue into the subject.

Once again the method is applicable to a wide variety of subjects, the primary use presently contemplated being human patients requiring transplant surgery, e.g., a replacement kidney, heart, lung, liver or intestine or replacement tissue derived from such organs or from other body tissues, e.g., pancreatic islet cells or bone marrow.

Although varying dosages of ultraviolet radiation may be employed for differing periods of time the presently preferred dose is one whose intensity is less than about 1000 $J/m^2$ and to which the subject is exposed for at least ten

minutes, e.g., twenty minutes. The irradiated organ or tissue may then be transplanted into the subject. The time within which the implant surgery is performed may vary as may the time during which the irradiated organ or tissue is stored prior to use, the limits depending primarily upon the nature of the organ or tissue. Generally, the irradiated organ or tissue will be employed within a few days after irradiation, e.g., within 24 hours after irradiation. The actual transplantation is performed using conventional procedures.

## EXPERIMENTAL DETAILS

### A. UV-irradiated Donor Specific Blood

The following experiment relating to transfusion with irradiated donor specific blood is also set forth in the August 19, 1983 issue of Science, volume 221, pages 754-756.

The successful transplantation of allogeneic pancreatic islets in mice depleted of cells bearing I region-associated antigens (Ia) by antiserum to Ia and the enhancement of skin allografts with this antiserum (1) suggest that allografted tissue depleted of Ia-bearing cells is accepted without being recognized as foreign. The type of Ia-bearing cell eliminated in such experiments is not known, but appears to be the dendritic cell; Ia-bearing dendritic cells are present in frozen tissue sections of islets and in the parenchyma of human kidneys, hearts, thyroid glands, and skin (2). Such a wide distribution suggests that the depletion of Ia-bearing cells from organ allografts may have clinical applicability not only to pancreatic islet

transplantation but to transplantation of other organs as well. Although abolition of the initial recognition of a foreign allograft by the host is critical to successful allografting without further immunosuppression, maintenance of a functioning allograft may depend on the initiation of donor-specific suppressor T lymphocytes in the host (3). Such a state of unresponsiveness to allogeneic tissue is seen when Ia-negative platelets and red blood cells are unable to provoke a primary immune response and attenuate the subsequent challenge with Ia-bearing cells (3).

This idea was further supported when treatment of diabetic mice with donor blood depleted of Ia-bearing cells allowed successful transplantation of fresh, untreated allogeneic islets of the blood donor strain (4). It appears, therefore, that immunization with Ia-negative donor blood cells induces immunological unresponsiveness to the donor strain in recipients by the stimulation of specific suppressor cells.

Our demonstration of a rapid and simple method of inducing donor-specific immunological unresponsiveness in adult animals that allows long-term survival of islet allografts is consistent with recent clinical studies in which donor-specific transfusions led to 1-year survival of kidney allografts in more than 90 percent of mismatched donor-recipient pairs of one haplotype (5). Since ultraviolet (UV) irradiation of the stimulating cell population in a primary mixed-lymphocyte reaction (MLR) leads to little or no proliferative response (6), we hypothesized that Ia-bearing cells may not need to be eliminated from blood before its use for immunization, but may need to be inactivated with UV light, leading to abrogation of the stimulating allogeneic signal while leaving major histo-

compatibility complex antigens intact for the induction of donor-specific immunological unresponsiveness.

Rats of strain ACI ($RT1^a$ were made diabetic with intravenous streptozotocin (60 mg/kg). A rat was used as a recipient of blood and islets only if its blood glucose concentration exceeded 300 mg/dl for more than 3 weeks. Islet allografts were considered to have been rejected when plasma glucose was greater than 200 mg/dl on two successive daily measurements.

Whole blood was obtained from normal Lewis rats ($RT1^l$) by intracardiac puncture. The blood was diluted 1:50 in phosphate-buffered saline (PBS), placed with a magnetic stirring bar into 250-ml petri dishes, and irradiated for 20 minutes with two Sylvania FS-20 lamps located 10 cm from the dishes. The blood cells were then centrifuged and the resulting pellet was resuspended in PBS to 50 percent packed cell volume. Each diabetic ACI rat received 1 ml of UV-irradiated blood or 1 ml of identically treated non-irradiated blood adjusted to 50 percent packed cell volume through the penile vein 3 weeks, 2 weeks, and 1 week before islet transplantation. One group of diabetic ACI rats received islets without previous transfusions.

Pancreatic islets were harvested from Lewis ($RT1^l$) and Wistar Furth (WF) ($RT1^u$) rats by the collagenase technique (7) and Ficoll gradient separation (8), with subsequent handpicking under a dissecting microscope. Some 1200 to 1500 freshly prepared allogeneic islets were transplanted intraportally into four groups of diabetic ACI rats. Two groups of islet recipients (groups 1 and 4) were first transfused with UV-irradiated whole blood. One control group (group 3) was not transfused before receiving islets, while a second control group (group 2) was transfused with

nonirradiated blood before allografting.

In vitro studies of Lewis rat peripheral blood lymphocytes that were either non-irradiated or irradiated in a manner identical to that of the whole blood were performed in conjunction with the above study (9). The binding of monoclonal antibodies to rat Ia (MRC-OX4) (10) and of a polyclonal rabbit antiserum to rat lymphocytes (M.A. Bioproducts) was determined with $^{125}$I-labeled staphylococcal protein A on Lewis rat lymphocytes obtained from peripheral blood that was UV-irradiated or untreated with irradiation (11). MLR's were performed by using Lewis lymphocytes obtained from irradiated or untreated whole blood (identical treatment to that described for transfusions before islet allografting) as stimulator cells and ACI thoracic duct lymphocytes as responders (12).

Lewis peripheral blood lymphocytes obtained from UV-irradiated blood did not stimulate ACI thoracic duct lymphocytes significantly compared to Lewis lymphocytes obtained from nonirradiated whole blood (Table 1).

Table 1. Effect of UV irradiation on the stimulatory activity of Lewis rat peripheral blood lymphocytes (PBL) in MLR's. Values are means ± standard deviations.

| Responder | Stimulator | [$^3$H]thymidine incorporation (count/min) |
|---|---|---|
| ACI | ACI | 465 ± 153 |
| ACI | Lewis PBL | 5371 ± 543 |
| ACI | Lewis PBL and UV irradiation for 20 minutes | 722 ± 102 |

In the radioimmunoassay there appeared to be no significant difference between lymphocytes obtained from irradiated Lewis peripheral blood and untreated blood (Table 2).

Table 2. Effect of UV light on the serological reactivity of Lewis rat PBL surface antigens. Values are mean counts (± standard deviations) of $^{125}I$-labeled staphylococcal protein A bound per assay (background, 200 count/min).

| Antigen | PBL | PBL and UV irradiation (20 minutes) |
|---|---|---|
| Rabbit antiserum to rat lymphocytes | 2996 ± 172 | 3315 ± 434 |
| Monoclonal antibodies to rat Ia (MRC/OX4) | 2050 ± 421 | 1963 ± 268 |

The rabbit antiserum to rat lymphocytes and the monoclonal antibody to rat Ia (MRC/OX4) showed similar binding to Lewis peripheral blood lymphocytes, regardless of whether UV irradiation was used. Therefore we did not detect allostimulation in the MLR's by peripheral blood lymphocytes that were irradiated, despite the clear demonstration by radioimmunoassay that major histocompatibility antigens are quantitatively unchanged by previous irradiation of lymphocytes.

In the in vivo allograft experiments, diabetic ACI recipients that were transfused with UV-irradiated Lewis whole blood and subsequently transplanted with fresh Lewis islets (group 1) showed 100 percent conversion to normo-

glycemia. There was no tissue rejection in any of the ten animals in a period of more than 160 days after allografting. The nontransfused control group (group 3) and the control group transfused with nonirradiated blood (group (2) had similar mean survival times (8.2 ± 2.9 and 8.8 ± 4.1 days, respectively. When third-party islets were transplanted into diabetic ACI recipients transfused previously with irradiated Lewis whole blood (group 4), rejection of islets and return to the diabetic state occurred in a normal fashion (mean survival time, 7.5 ± 3.0 days) (Table 3 and FIG. 1).

Table 3. Survival of islet transplants in the various treatment and control groups.

| Group | Treatment | Donor strain | N | Survival time (days) | Mean survival time ± standard deviation (days) |
|---|---|---|---|---|---|
| 1 | Irradiated Lewis blood | Lewis | 10 | >160 | |
| 2 | Lewis blood | Lewis | 5 | 3, 7, 9, 11, 14 | 8.8 ± 4.1 |
| 3 | None | Lewis | 5 | 5, 7, 8, 8, 13 | 8.2 ± 2.9 |
| 4 | Same as group 1 | WF | 4 | 6, 6, 6, 12 | 7.5 ± 3.0 |

These results demonstrate that transfusions of UV-irradiated whole blood of the donor type lead to a prolonged and perhaps indefinite survival of islet allografts and induction of normoglycemia in the diabetic host. These results are obtained without any immunosuppressive drugs, and the induction of unresponsiveness with UV-irradiated blood appears to be donor-specific. Parallel in vitro studies suggest that UV irradiation of blood abrogates the allostimulatory effect of blood lymphocytes in the MLR—even in the presence of UV-absorbing red blood cells——while not affecting the serological reactivity of Ia (as shown by radioimmunoassay) or antigens detected by rabbit antiserum to rat lymphocytes. These findings suggest that allostimulation requires the presence of a metabolically active Ia-bearing cell and that immunization with inactivated cells can lead to subsequent strain-specific immunological unresponsiveness to islet allografts in rats. Although immunological unresponsiveness to allografts and induction of T suppressor cells have been demonstrated with various blood transfusion protocols, the results were inconsistent and immunosuppression was generally required (13). We believe, as Faustman et al. (4) suggested, that in islet transplantation sensitization and subsequent rejection occurs because of "contaminating" Ia-bearing cells; however, our findings suggest that these cells need not be physically eliminated but may simply be inactivated to result in immunological unresponsiveness of the host without the need for further immunosuppressive intervention. Studies of recombinant mouse strains (14) support the conclusion that the Ia signal is altered by UV irradiation without a significant change in the class I antigens. This may occur through metabolic inactivation of the cell.

Thus, UV irradiation offers a promising method for the

induction of donor-specific immunological unresponsiveness. The use of UV irradiation for immunological inactivation of blood products could be easily applicable to allotransplantation in other species for which specific antibodies to Ia are not available or required. This approach may prove useful in the transplantation of human organs, an area where donor-specific blood transfusions are already in use, and may eliminate the possibility of sensitization to major histocompatibility antigens of the donor. Prolonged (or indefinite) islet allograft survival and correction of diabetes may be achieved by this simple maneuver without requiring immunosuppression of the diabetic host.

B. <u>UV-irradiated Organs and Tissues</u>

Although major histocompatibility complex (MHC) disparity causes rejection of grafted tissue, the recognition by the host of this incompatibility appears to be the critical factor in initiating the rejection process. The recognition of foreignness by the host appears to require both the pressence of Class I and Class II MHC antigens on the graft and lympho-reticular cells bearing both classes of antigens are thought to be responsible for sensitizing the host toward a primary immune response (15-17). We present data that ultraviolet (UVB) irradiation, at an appropriate dose which can abrogate an MLC response after irradiation of rat dendritic stimulator cells (18-21), can also attenuate the immunogenicity of pancreatic islets without alteration of its endocrine function and prolong rat islet allograft survival in diabetic hosts without the use of immunosuppressive agents.

The exact nature of the 'passenger leukocyte' which has been implicated in causing graft rejection is not clear.

The rat dendritic cells have been demonstrated to be extremely powerful as accessory cells in T cell proliferation and in causing acute rejection of otherwise 'passenger leukocyte'-depleted rat kidneys (22). We first investigated the ability of UV irradiation of rat dendritic cells, derived from afferent lymph, to attenuate their stimulatory activity in an MLC. Using ACI ($RT1^a$) rat thoracic duct lymphocytes (TDL) as responders and Lew ($RT1^l$) rat dendritic cells (DC) as stimulators, a high stimulation index (SI) of >400 was obtained with $10^5$ DC (FIG. 2). When the number of DC stimulators were decreased to $125x10^5$ the SI remained markedly elevated (162).

Dendritic cells, UV irradiated (Sylvania FS-20 at exposures of 800 $J/m^2$ to 1000 $J/m^2$) were completely ineffective as stimulators in the MLC with resulting SI of 3. Although dendritic cells are extremely powerful allogeneic stimulators as demonstrated in the MLC (20, 23) and in causing graft rejection (21), they appear to be inactivated by UV irradiation but not by gamma irradiation.

Once the dose range of UV irradiation necessary to attenuate the MLC response was defined, we exxamined the ability of UV irradiated islets (same dose range) to reverse the diabetic state in syngeneic streptozotocin (STZ)-induced diabetic rats (FIG. 3). Lewis rat islets irradiated with 1000 $J/m^2$ and subsequently transplanted intraportally into diabetic Lewis recipients converted diabetic animals to a normoglycemic state for less than 5 days; islets irradiated with 1100 or 1200 $J/m^2$ failed to convert them. Irradiation (UV) with 600 or 900 $J/m^2$ resulted in indefinite conversion to normoglycemia in all diabetic syngeneic recipients. Thus, the UV irradiation dose that can abrogate the proliferative response in the MLC using $10^5$ DC as stimulators, has no deleterious effect on the <u>in vivo</u> endocrine function

of syngeneic islet grafts irradiated with 900 $J/m^2$.

To determine whether the immunogenicity of allogeneic islets was reduced after such irradiation, Lewis ($RT1^l$) islets were transplanted into STZ-induced diabetic ACl rats (FIG. 4, Table 4).

Table 4

The Effect of Direct UV Irradiation on

Lewis Islet Allograft Survival in Diabetic ACI Recipients

| Islet Treatment | n | Survival Time (Days) | MST ± SD |
|---|---|---|---|
| 24 hour culture | 10 | 4, 4, 4, 6, 6, 7, 7, 8, 9, 13 | 6.8 ± 2.7 days |
| UV & 24 hour culture | 11 | 10, 10, 18, 75, 75, 75, 75, 110, 110, 110, 110 | >80 days |

All the control ACI animals receiving Lewis islets cultured for 24 hours at 37°C rejected their grafts and became diabetic again at 6.8 ± 2.7 days. When Lewis islets were exposed to 900 J/$m^2$ of UV irradiation, cultured for 24 hours and then transplanted into diabetic ACI recipients, islet survival was prolonged in 8 of 11 transplanted animals to more than 70 days (4>110 days) with all 8 still normoglycemic. These results indicate that treatment of allogeneic rat islets with UV irradiation prior to allotransplantation, at an exposure that is not deleterious to its endocrine function, can reduce the islets' immunogenicity and permits prolonged allograft survival without immunosuppression.

The importance of passenger leukocytes in initiating allograft rejection has been a recurring theme in transplantation-immunology (24-27). In the transplantation of islets various <u>in vitro</u> culture techniques (28, 29) have been used to prolong allograft survival, all relying on the presumption that long-term cultures selectively deplete the islet of lympho-reticular elements (30). More recently, the use of anti-Class II MHC antigen sera and complement to remove Ia bearing cells have permitted prolonged allogeneic islet graft survival in the mouse (31). The exact nature of passenger leukocytes involved is uncertain. This question has been recently approached by studies of the role of interstitial dendritic cells in the primary alloactivation of T cells in MLC and their role in the rejection of rat kidney allografts (20, 22, 32). The ubiquitous presence of such Class II MHC antigen bearing cells identified in rat islets, kidneys and hearts would suggest that these cells may be the putative 'passenger leukocytes' responsible for direct sensitization of the host toward the allograft antigen (21, 33). In this study, we have demonstrated that dendritic cells are extremely powerful acti-

vators of relevant T responder cells in the primary MLC, and that their stimulatory activity can be completely inactivated with appropriate UV irradiation.

Many previous studies have shown that UV irradiation has a selective effect on antigen presenting cells (APC) (34-36) and that passive transfer of UV irradiated APC can induce antigen specific T suppressor cells (37). These and other studies (38-40) would therefore suggest that improper antigen presentation, i.e., allograft without stimulatory leukocytes may induce preferentially production of T suppressor cells or effect nonrecognition of foreignness until antigens are represented to host T cells by host APC's whereby production of donor-specific T suppressor cells may also occur. We have previously shown that UV irradiation of peripheral blood lymphocytes does not quantitatively alter cell surface antigens including Class II MHC antigens (41). It would appear that primary allostimulation not only requires Class I and Class II antigen bearing lympho-reticular cells (31, 42), but that they must be metabolically active and are susceptible to inactivation by UV irradiation (43).

The prolonged survival of irradiated islets suggests that their exposure to UV irradiation that is effective in abrogating the MLC response, is selectively effective in attenuating the stimulatory activity of the interstitial dendritic cells or other allostimulatory cells present in the islet preparation. Since islets are not single cell suspensions, some allostimulatory leukocytes may escape complete inactivation which might explain a 27% failure rate in prolongation of islet allograft survival. A more precise quantitative method of delivery of UV irradiation and elucidation of its ultimate effect on islet function and simultaneously on the allostimulatory activity of the

dendritic and other lympho-reticular cells contained in the islet preparation, is necessary before this approach is uniformly successful.

In conclusion, we have demonstrated that brief UV irradiation of islets can reduce their immunogenicity without affecting their endocrine function and permit long-term islet allograft survival and function in diabetic hosts without the use of any immunosuppressive agents. This approach offers distinct advantages over long-term culture techniques and does not require the use of specific anti-Class II MHC antigen sera. We believe that this study forms the basis for islet transplantation in other animals and ultimately in man.

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WHAT IS CLAIMED IS:

1. A method for enhancing acceptance of transplanted organs or tissues in a subject which comprises treating donor specific blood with a suitable dose of ultraviolet radiation for an appropriate period of time, transfusing the irradiated blood into the subject during a suitable period of time prior to transplanting the organ or tissue into the subject and then transplanting the organ or tissue into the subject.

2. A method according to claim 1, wherein the subject is a human being.

3. A method according to claim 1, wherein the organs or tissues are or are derived from kidney, heart, lung, liver, intestine or bone marrow.

4. A method according to claim 1, wherein the suitable dose is less than about 1000 $J/m^2$.

5. A method according to claim 1, wherein the appropriate period of time is greater than about ten minutes.

6. A method according to claim 1, wherein the suitable period of time during which the irradiated blood is transfused into the subject is more than about one week prior to transplantation and comprises more than one transfusion.

7. A method according to claim 6, wherein the suitable period of time during which the irradiated blood is transfused into the subject comprises transfusions at three, at two and at one week prior to transplantation.

8. Donor-specific blood irradiated with a suitable dose of

ultraviolet radiation.

9. Donor-specific blood according to claim 8, wherein the suitable dose is less than about 1000 $J/m^2$.

10. A subject treated according to the method of claim 1.

11. A method for enhancing acceptance of transplanted organs or tissues in a subject which comprises treating the organ or tissue to be transplanted with a suitable dose of ultraviolet radiation for an appropriate period of time and then transplanting the irradiated organ or tissue into the subject.

12. A method according to claim 11, wherein the subject is a human being.

13. A method according to claim 11, wherein the organ or tissue to be transplanted is or is derived from kidney, heart, lung, liver, intestine or bone marrow.

14. A method according to claim 13, wherein the organs or tissues are pancreatic islet cells.

15. A method according to claim 1, wherein the suitable dose is less than about 1000 $J/m^2$.

16. A method according to claim 1, wherein the appropriate period of time is greater than about ten minutes.

17. An organ or tissue irradiated with a suitable dose of ultraviolet radiation.

18. Pancreatic islet cells irradiated with a suitable dose of ultraviolet radiation.

19. An organ or tissue according to claim 17, wherein the suitable dose is less than about 1000 $J/m^2$.

20. A method of preparing an organ or tissue according to claim 17 which comprises treating the organ or tissue with a suitable dose of ultraviolet radiation for an appropriate period of time.

21. A subject treated according to the method of claim 11.

FIG. 1

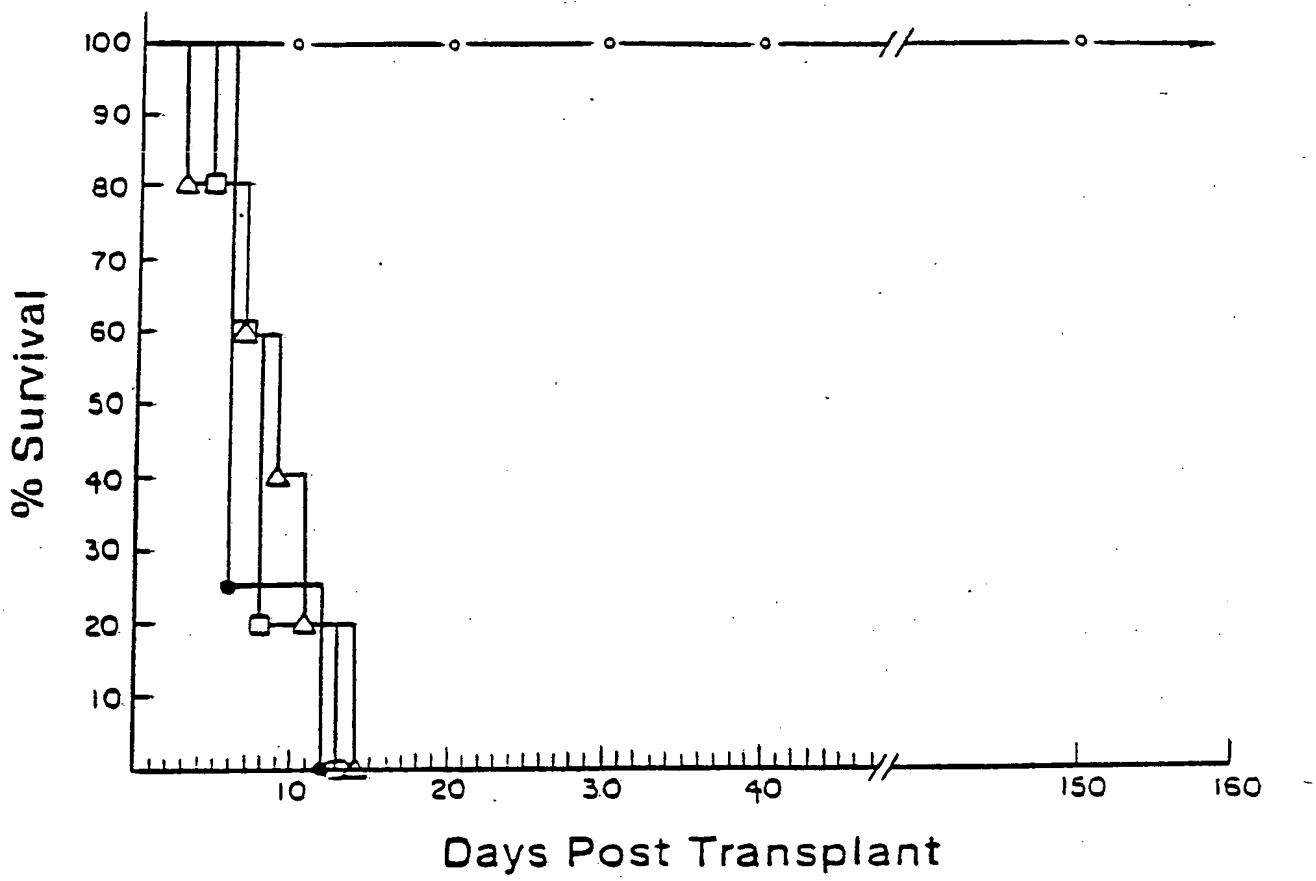


FIG. 2

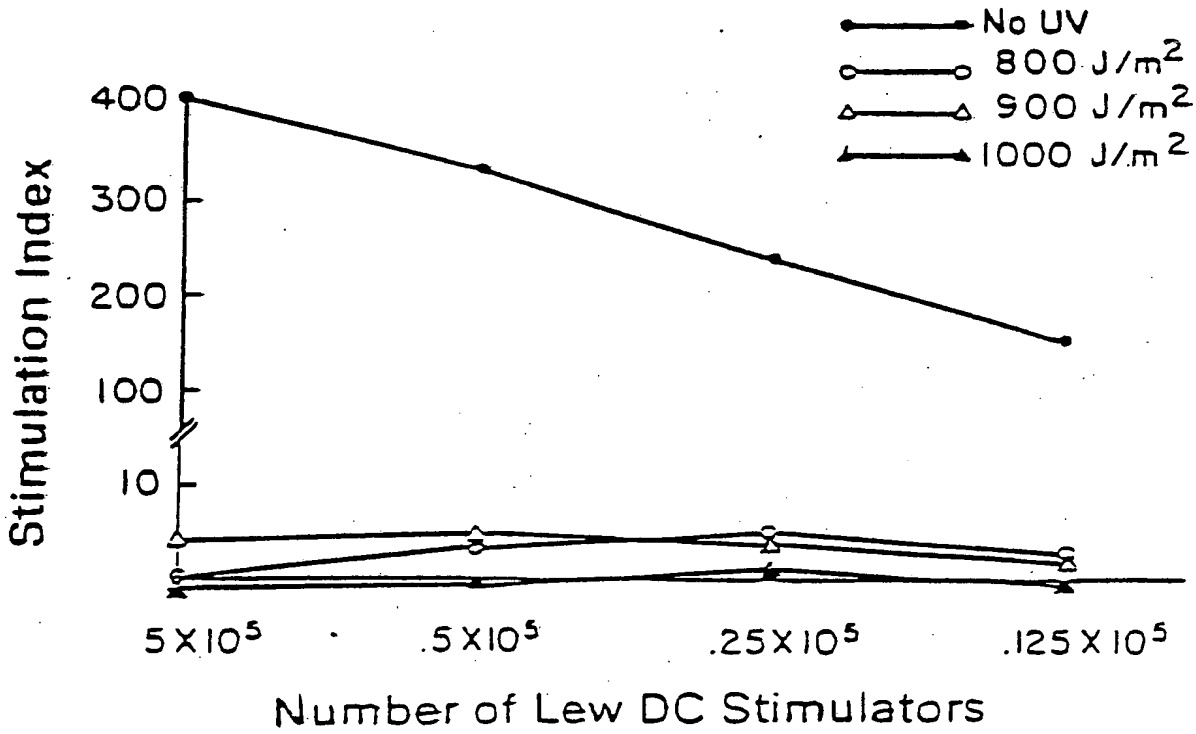


FIG. 3

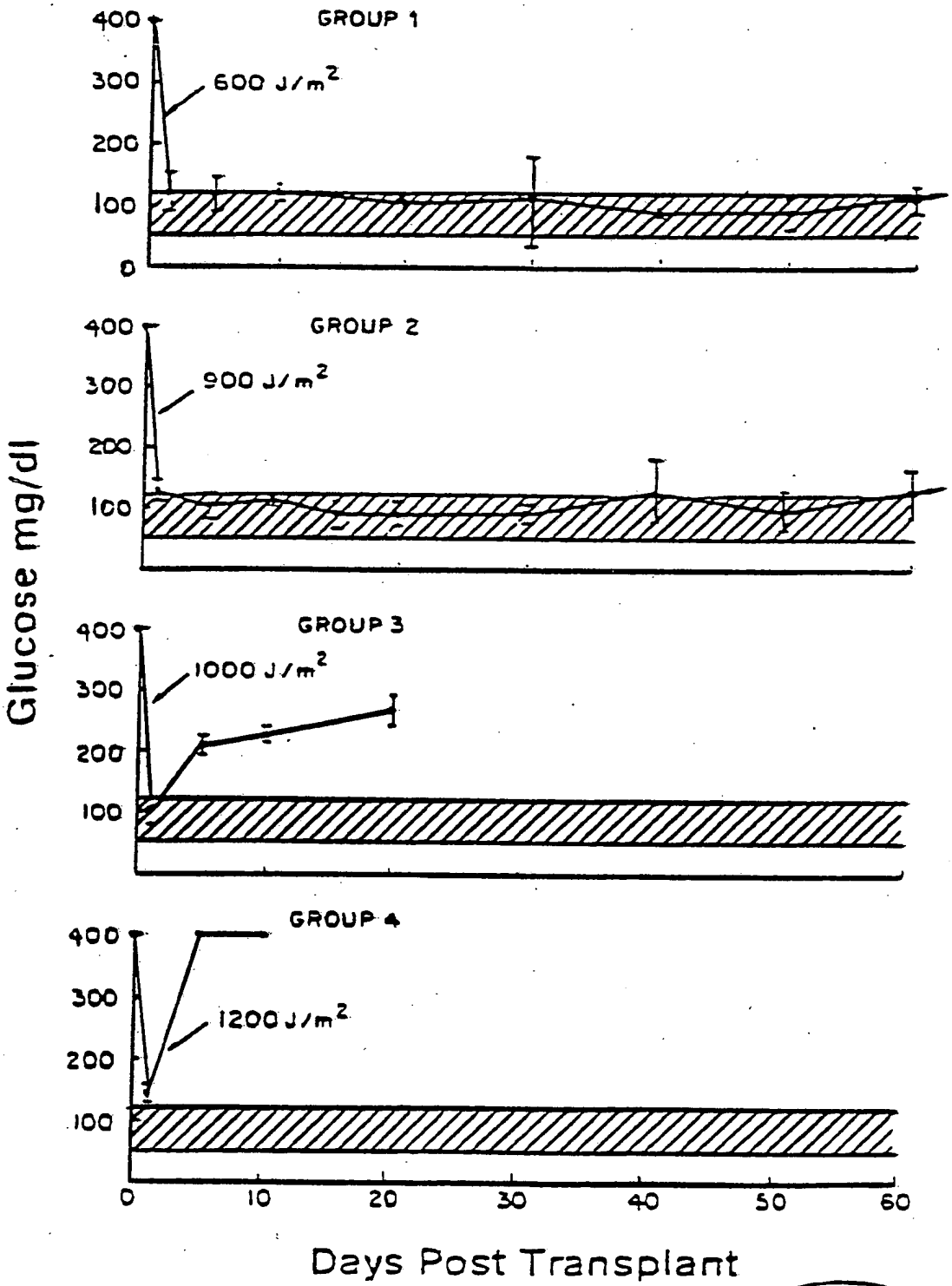
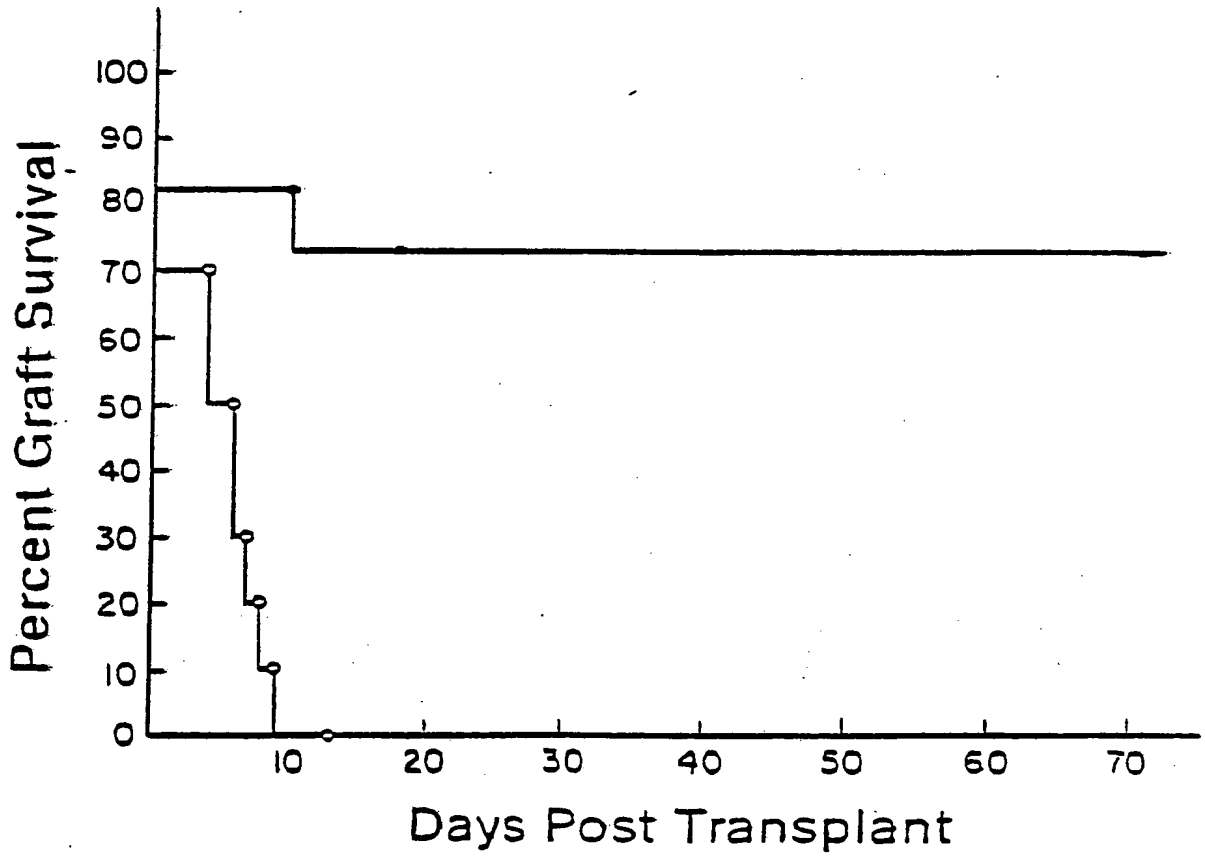


FIG. 4



# INTERNATIONAL SEARCH REPORT

International Application No PCT/US84/01347

## I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl? A01N 1/02; C12N 13/00; A61M 37/00,31/00; A61N 1/30,5/06;A61K 35/12,14
U.S. 435/1,2,173,283;604/4,20,49,52,905;128/395;424/95,101,204/158S,157.1S

## II. FIELDS SEARCHED

**Minimum Documentation Searched [4]**

| Classification System | Classification Symbols | |
|---|---|---|
| U. S. | 435/1,2,173,283<br>604/4,20,49,52,905<br>424/95,101 | 128/395<br>204/158S, 157.1S |

**Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]**

Lexpat Search
Computer Search of Chemical Abstracts, Biosis, Previews & Medline Databases

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| X | US,A, 1,883,877, published 11 September 1928 Edslom et al. | 8,9 |
| X | US,A, 3,973,001, published 03 August 1976 Jaeger et al., see col. 1, lines 14-21 | 8,9 |
| X | SU,A, 342630 , published 18 July 1972 | 8,9 |
| Y,P | US,A, 4,456,589, published 26 June 1984 Holman et al., see col. 2, lines 6-15 | 11-21 |
| X | N, Anatomy and Physiology, published 1961, Kimber et al., The MacMillan Company, New York, pages 306-315, 430-431 | 17-19 |
| Y | US,A, 1,883,877, published 11 September 1928 Edslom et al. | 1-7, 10-21 |

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| 19 November 1984 | 26 NOV 1984 |
| **International Searching Authority [1]** | **Signature of Authorized Officer [20]** |
| ISA/US | Randall E. Deck |

Form PCT/ISA/210 (second sheet) (October 1981)

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

| | | |
|---|---|---|
| Y | US,A, 3,973,001, published 03 August 1976 Jaeger et al., see col. 1, lines 14-21 | 1-7, 10 |
| Y | SU,A, 342630 , published 18 July 1972 | 1-7, 10 |
| A | US,A, 4,321,918, published 30 March 1982 Clark II | 1-21 |
| X | US,A, 4,321,919, published 30 March 1982 Edelson | 8,9 |
| Y | US,A, 4,321,919, published 30 March 1982 Edelson | 1-7, 10 |

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers_____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers_____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

**VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]**

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, 16 with indication, where appropriate, of the relevant passages 17 | Relevant to Claim No 18 |
|---|---|---|
| X | DT,A, 3,109,691, published 23 September 1982 | 8,9 |
| Y | DT,A, 3,109,691, published 23 September 1982 | 1-7, 10 |
| A,E | US,A, 4,471,629, published 18 September 1984 Toledo-Pereyra | 10-21 |
| X | DT,A, 2803446 , published 02 August 1979 | 17,19 |